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PHYTOPATHOLOGY

ELECTRON MICROSCOPE OBSERVATIONS ON THE FLAGELLA OF THE ZOOSPORES OF *PHYTOPHTHORA INFESTANS*

BY

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(Communicated by Prof. A. J. P. OORT at the meeting of June 27, 1959)

Introduction

In observing by a phase-contrast microscope the living zoospores of *Phytophthora infestans* (MONT.) DE BARY, the endpieces of the flagella were found to have paddle-like structures. This character was further investigated by observing zoospores continuously, from the moment of emergence from the sporangium. The results of these observations can be described as follows:

After swimming in a water mount at room temperature for some minutes, a zoospore suddenly produces a swelling at the longest flagellum near the place of insertion. Subsequently this swelling moves to the end of the flagellum. Mostly this first swelling is followed by a second and sometimes even by a third. In the meantime the short flagellum also develops a swelling. Sometimes it can be observed that in flagella with a swelling about halfway their length the endpiece turns over and settles down on the rest of the flagellum. Ultimately all the swellings concentrate in a paddle-like structure at the tip of the flagellum. Presently the flagellum is hidden from the eyes of the observer by its settling against the body of the zoospore.

Different from this development sometimes a flagellum can suddenly be seen becoming detached from the zoospore in one of the described stages at the place of its insertion and then floating away. The body of the zoospore which is kidney-shaped at first, gradually becomes globular. The whole process (fig. 1) takes 1–2 minutes.

When the observations are continued it is found that at last all zoospores burst instead of producing a germ tube as is the case under natural conditions. As the enlargement which can be obtained by means of a phase-contrast microscope is too small to use it for an explanation of the phenomenon, an attempt has been made to obtain more information with the aid of an electron microscope.

Methods

Zoospores were obtained by placing sporangia at 10° C for three hours in a petri dish with glass-distilled water. A drop of the zoospore suspension

obtained in this way was transferred to a slide. Then the zoospores were killed and fixed by placing the droplet in contact with vapour from a 2-percent solution of osmic acid for 1/2–2 minutes. At first the difficulty was experienced that the zoospores burst at the moment of drying up.

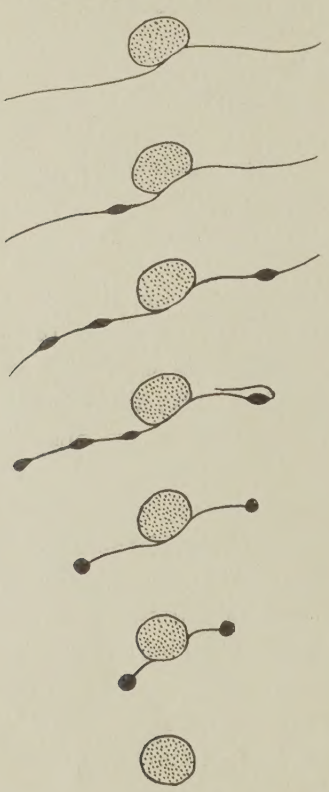

Fig. 1.
Successive stages in the development of paddle-like structures on the flagella.

This could be largely obviated by adding a small quantity of detergent to the zoospore suspension. By means of a micropipet a droplet of the suspension was then transferred to the electron microscope carriers. After drying in the laboratory the preparations were examined provisionally by means of a phase-contrast microscope. For this purpose the microscope carriers were placed with the downward bent lip along the edge of a slide. The best preparations were destined for further investigations; for this we had a Philips electron microscope, type for 100 KV, at our disposal.

Results

A general view of a zoospore (fig. 2) shows that one of the two flagella is covered with hairs and has an obtuse tip (the tinsel-type flagellum) whereas the other one is hairless and has a tapering endpiece (the whiplash-type flagellum). From a greater enlargement of the tinsel-type flagellum it appears that the hairs are borne singly in two rows down the sides of

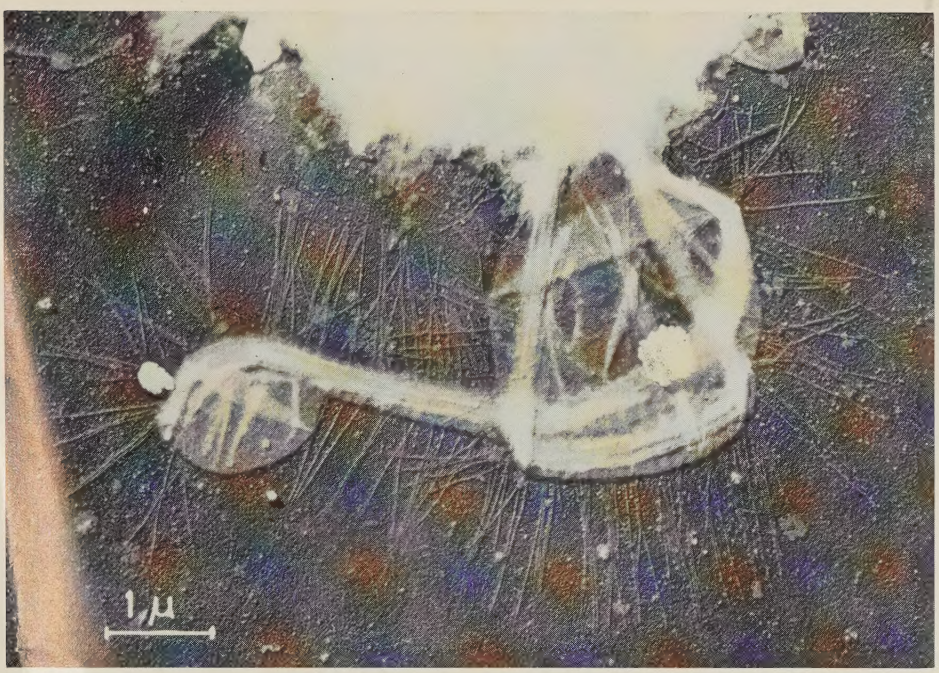

Fig. 8. Electron micrograph of a tinsel-type flagellum showing the membranous structure of the "swellings".

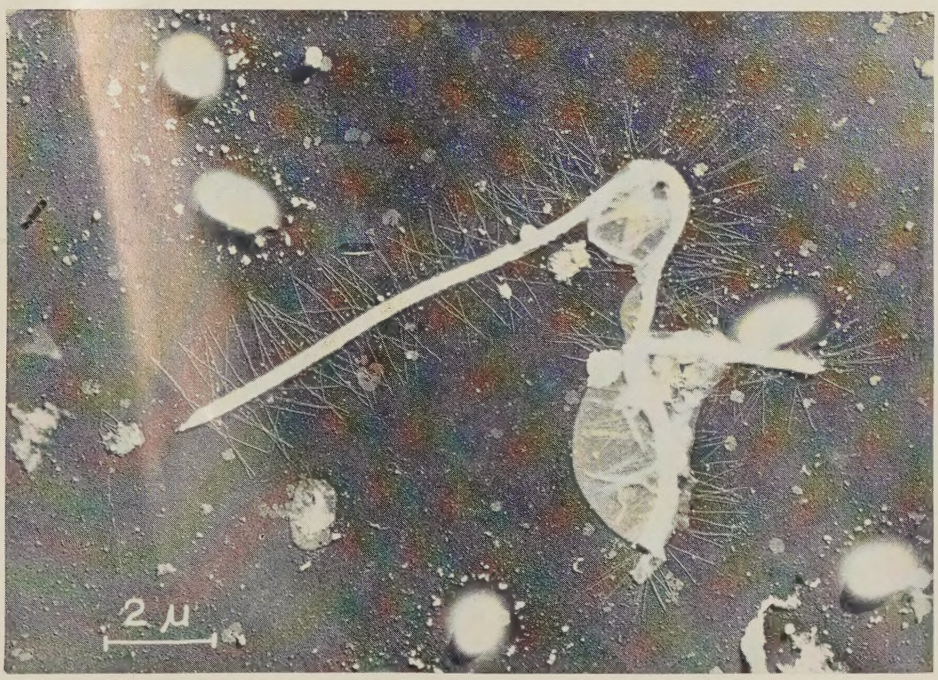

Fig. 9. Two entangled tinsel-type flagella comparable to that of fig. 8.

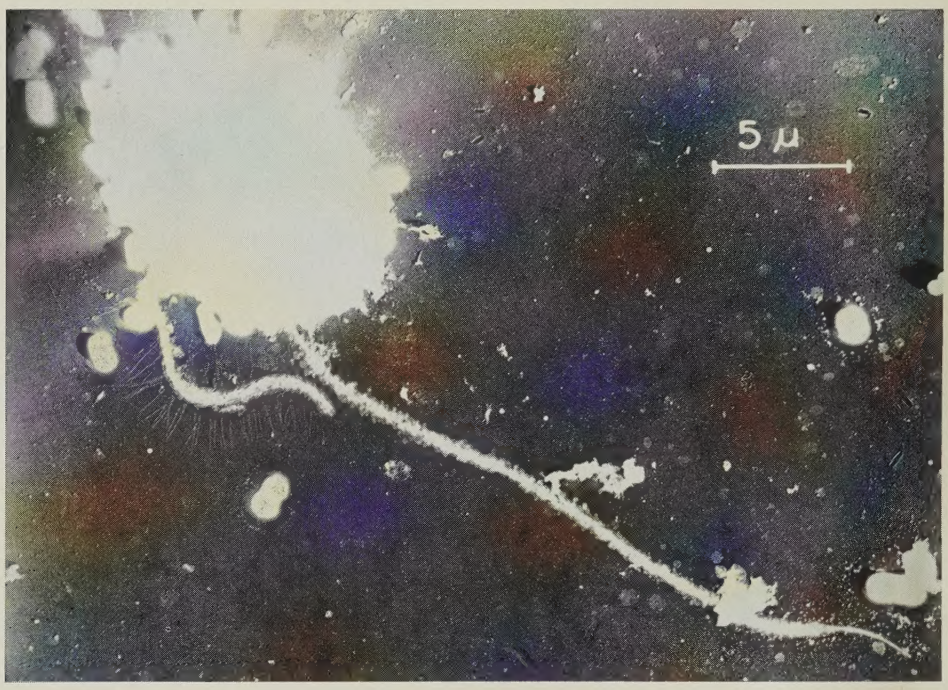

Fig. 2. A zoospore showing the complete whiplash-type flagellum and a part of the tinsel-type flagellum. Electron micrograph.

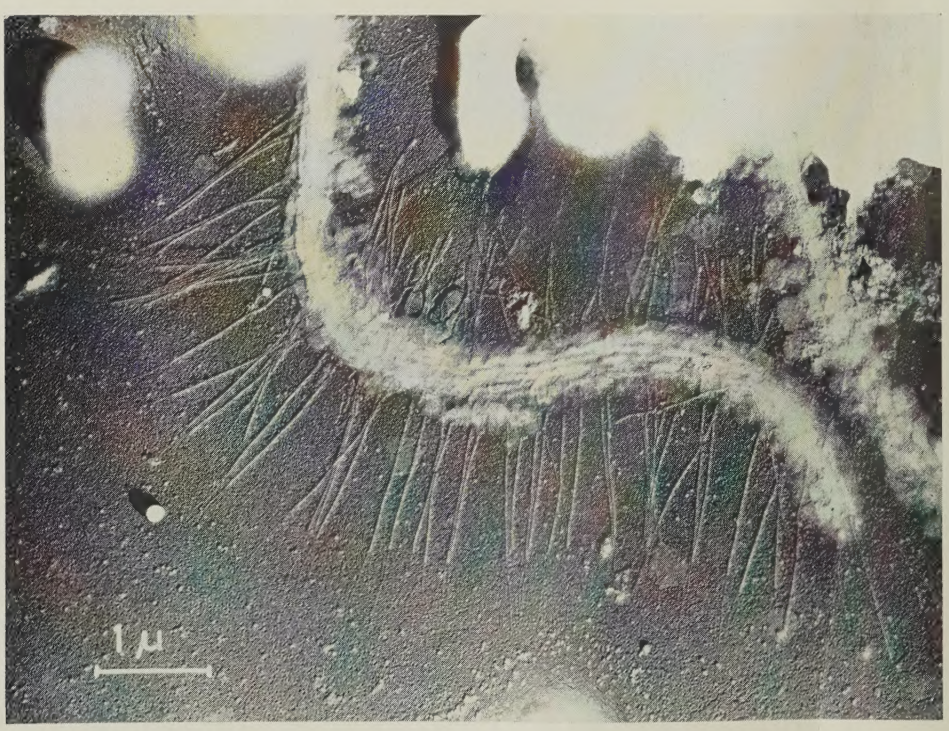

Fig. 3. Enlarged view of the tinsel-type flagellum of the zoospore of fig. 2. Note the hair-points at the end of the cilia. Electron micrograph.

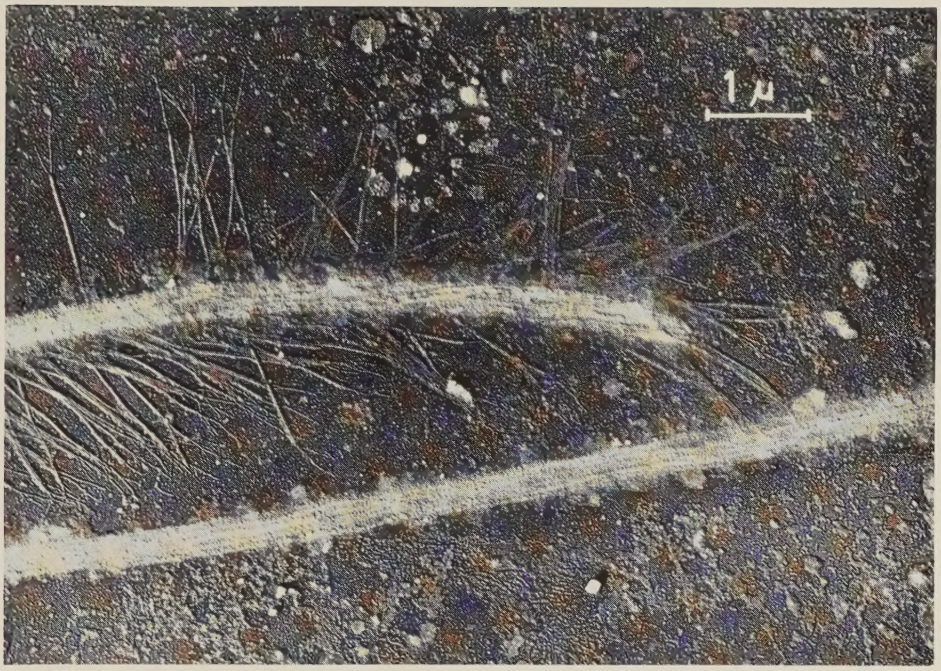

Fig. 4. Part of a whiplash- and of a tinsel-type flagellum showing a striated axis. Electron micrograph.

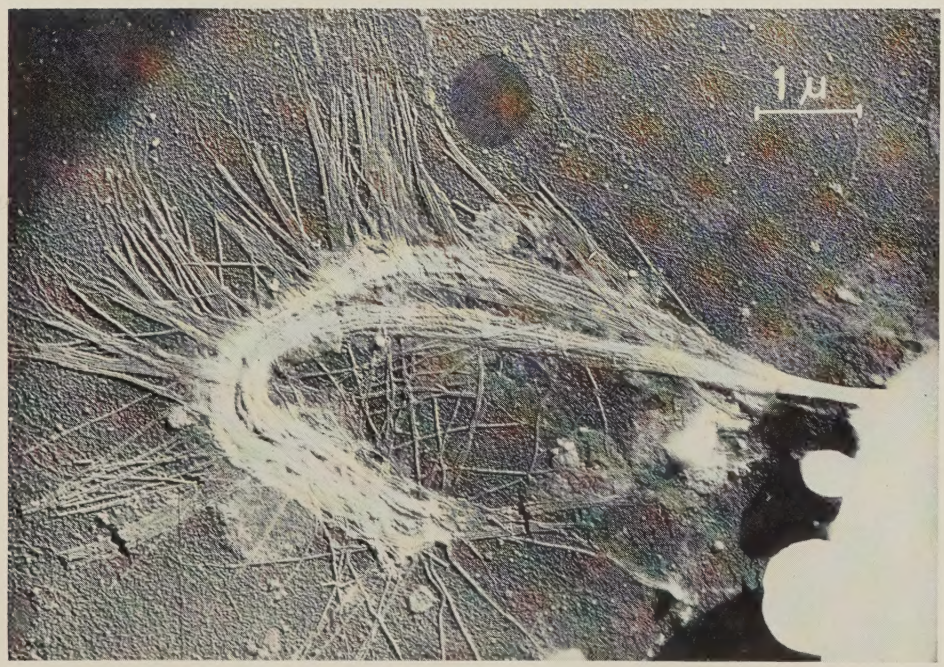

Fig. 5. Partly disintegrated tinsel-type flagellum. Electron micrograph.

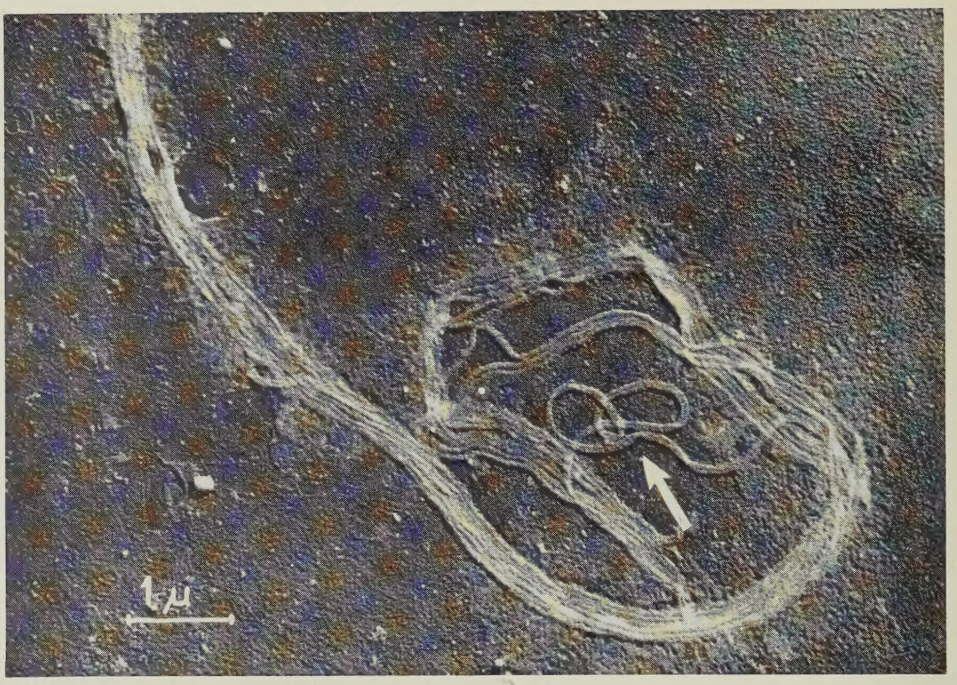

Fig. 6. Whiplash-type flagellum showing fibrillar disintegration. Note the displaced central pair (arrow) of strands and the opened and flattened cylinder of nine outer strands. Electron micrograph.

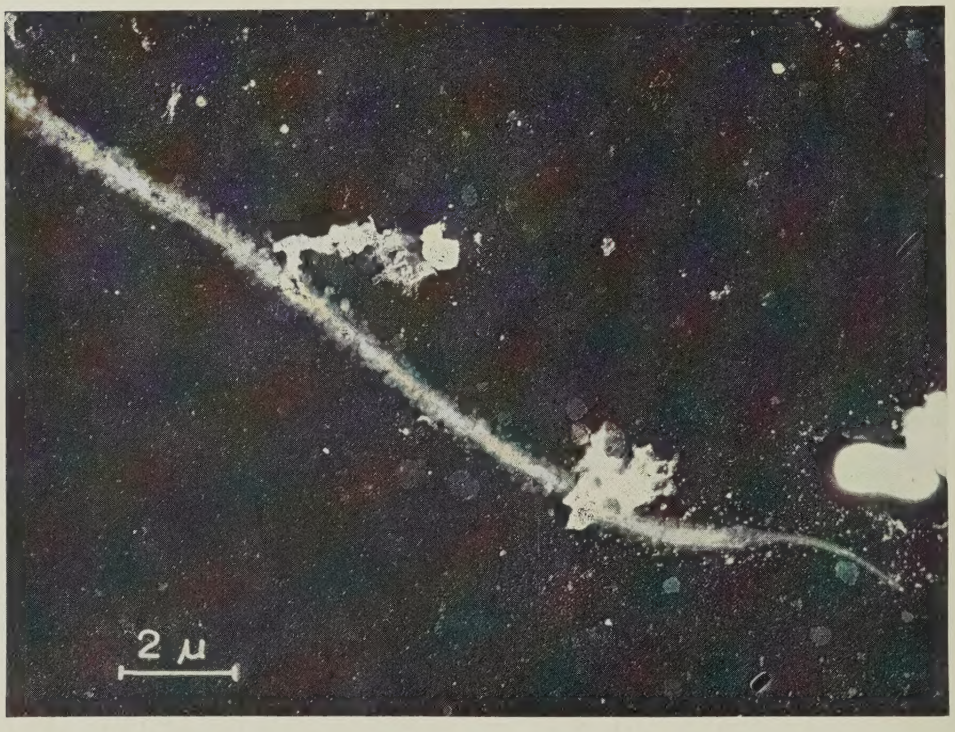

Fig. 7. A whiplash-type flagellum showing reduction in number of strands in the formation of the whiplash. Electron micrograph.

the flagellum (fig. 3). They consist of two parts, viz. a basal part, covering 3/4th of the entire length, and a thin hair-point. The body consists of a core and an outer membrane or sheath. The core exhibits a fibril structure (figs. 3 and 4). As appears from fig. 5 the hairs of the tinsel-type flagellum originate from the axis. A picture (fig. 6) of a much disintegrated flagellum shows that the core consists of eleven strands. The cracknel in the centre of the cluster (see arrow) consists of two adjacent strands which can be concluded from the presence of a groove running lengthwise. Presumably there are two central strands surrounded by nine others. This has also been described by other workers. In many flagella the sheath has been reduced to a granular residue.

From a greater enlargement of the whiplash-type flagellum shown in fig. 2 (fig. 7) it is apparent that the majority of the strands end where the tapering endpiece begins; presumably only the two central fibrils continue into the tip. In the tinsel-type flagellum contrarily all strands continue into the obtuse endpiece (fig. 3).

In various electron microscope pictures structures occur which may be related with the paddle-like structures of the flagella observed *in vivo* (figs. 8 and 9). At the end of the tinsel-type flagellum in fig. 8 a swelling can be observed. The upper side of this swelling is formed by the curved axis of the flagellum, the rest being a membrane as appears from the presence of folds. In the cluster of flagella in the same figure the presence of the membrane can be clearly seen. The same holds for the swellings of the two entangled tinsel-type flagella pictured in fig. 9. At the nod situated in fig. 9 at the top right the impression is given that the protruded sheath has caused the flagellum to curve. Fig. 8 shows that the sheath is still present in the part of the flagellum between the two swellings. At the place where the membrane is seen the sheath cannot be distinguished. From this it is concluded that the paddle-like structures are caused by protrusion of the sheath which presumably arises from a pressure of some fluid present within the sheath. Considering the fact that in later stages the sheath disappears, the phenomenon is ascribed to beginning disintegration of the flagella.

Discussion

Paddle-like structures at the tips of flagella have drawn the attention of various research workers (Ferris, 1954; Katsura *et al.*, 1956; Santilli, 1958). Ferris, on the basis of observations with the electron microscope, comes to the conclusion that the swellings are produced by coiling of the flagella. However, the mere fact of rolling up cannot sufficiently account for the paddle-like structures since also in the pictures gives by Ferris in the swellings a substance with a membranous structure can be already distinguished. The findings of Katsura *et al.* are based on investigations with the phase-contrast microscope. Their description (according to the summary) and drawings are in good agreement with

the results of our own investigations. However, the above authors also explain the paddle-like structures by a rolling up of flagella. SANTILLI, in investigations by means of an electron microscope, comes to the conclusion that in *Plasmopara viticola* the swellings are not caused by a coiling of the flagellum but by local accumulations of disintegrated material. This explanation very well agrees with the results of our examinations.

The nod in the flagella of fig. 9 upper right is highly similar to figure 16 of MANTON *et al.* (1951) of a flagellum of *Saprolegnia ferax*. However, their explanation is the following: "The curvature of the axis, induced presumably by surface tension exerted by the material of the sheath, is very conspicious.....". The results of our own observations are that the protruded sheath causes the flagellum to curve.

The observation that the hairs of the tinsel-type flagella consist of two parts is a confirmation of both FERRIS' findings for *Phytophthora infestans* and the findings of MANTON *et al.* (1951) for *Saprolegnia ferax*.

The internal structure of the flagella also has been the subject of investigations (MANTON, 1952, 1953; KOCH, 1956; KOLE, 1957). In various organisms it has been established that the flagella consist of strands and that the number and arrangement of these strands are always the same. In most cases eleven strands were found, a central pair being surrounded by nine others. The fibril bundles are enclosed in a membrane and in some cases an internal structure with spiral form has been observed between the peripheral fibrils and the core. There are indications that the separate fibrils again are built up by subfibrils (KOCH, 1956; KOLE, 1957). Our own investigations on *Phytophthora infestans* have produced no evidence of a finer structure of the strands.

The opinions on the structure of the whiplash differ considerably (KOCH, 1956). The results of our own investigations both concerning *Synchytrium endobioticum* (KOLE, 1957) and *Phytophthora infestans* are in close agreement with KOCH's findings (1956) for *Rhizophidium sphaerotheca*, viz. that the central pair of fibrils run through into the end of the flagellum, while the nine other fibrils are shorter and are not all of the same length.

Summary

By means of a phase-contrast microscope paddle-like structures were observed on the flagella of swimming zoospores of *Phytophthora infestans*. The results of observations with an electron microscope suggest that these paddle-like structures are protrusions of the sheath of the flagella. The hairs of the tinsel-type flagellum consist of two parts viz. a basal part covering 3/4th of its total length and a thin endpiece. The flagella consist of eleven strands enveloped by a sheath. Analogous to other cases there are presumably two central strands surrounded by nine other strands.

Acknowledgment

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REFERENCES

FERRIS, V. R., A note on the flagellation of *Phytophthora infestans* (MONT.) DE BARY, Science **120**, 71–72 (1954).

KATSURA, K., K. HARADA and M. MURAKAMI, The flagella of zoospores of *Phytophthora capsici* LEONIAN as viewed with the phase contrast microscope. Ann. Phytopath. Soc. Japan **21**, 71–73 (1956).

KOCH, W. J., Studies of the motile cells of chytrids. I. Electron microscope observations of the flagellum, blepharoplast and rhizoplast. Am. J. Bot. **43**, 811–819 (1956).

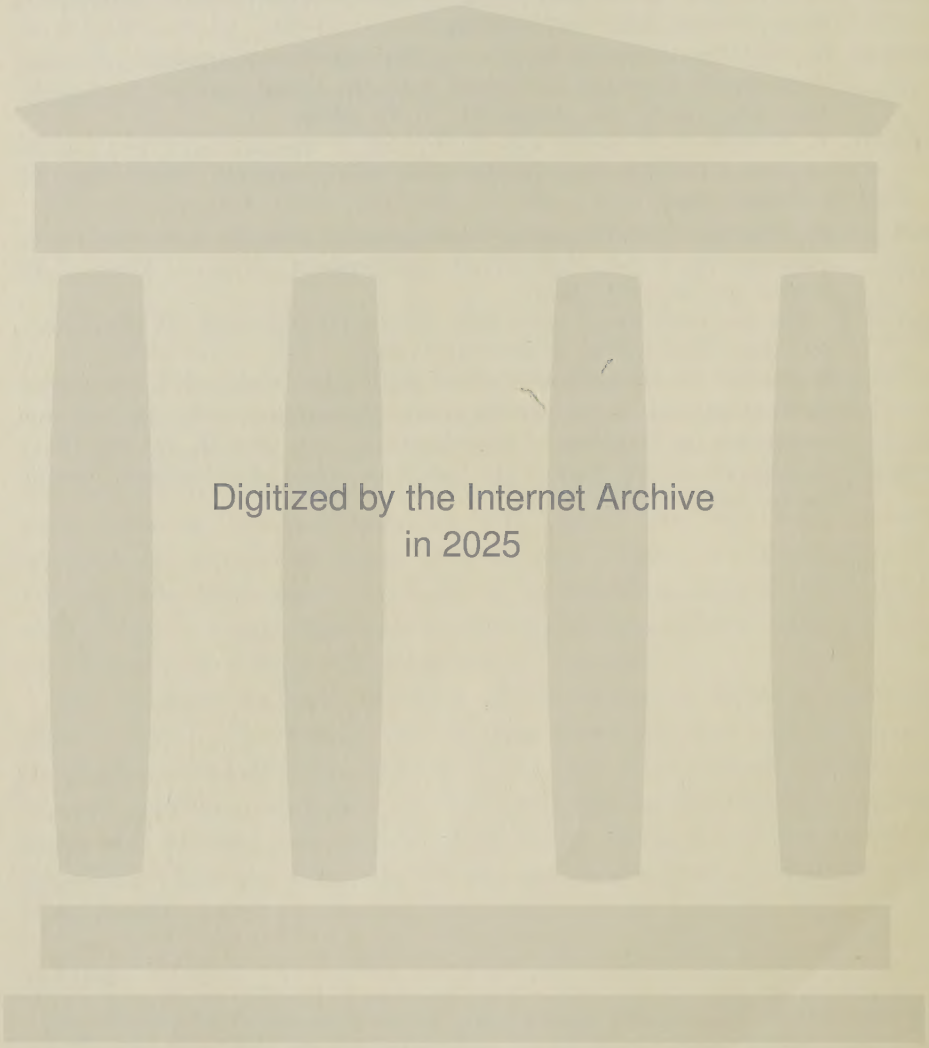
KOLE, A. P., Elektronenmicroscopische waarnemingen over de zoösporen uit de zomersporangia van *Synchytrium endobioticum* (SCHILB.) PERC. T.Pl. ziekten **63**, 361–364 (1957).

MANTON, I., The fine structure of plant cilia. Structural aspects of cell physiology. Soc. exp. Biol. Symp. **6**, 306–319 (1952).

————, Number of fibrils in the cilia of the green algae. Nature **171**, 485 (1953).

————, B. CLARKE and A. D. GREENWOOD, Observations with the electron microscope on a species of *Saprolegnia*. J. exp. Bot. **2**, 321–331(1951).

SANTILLI, V., Zoospore morphology of the California strain of *Plasmopara viticola*. Nature **181**, 924–925 (1958).



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